

TREASURY DEPARTMENT.

Public Health and Marine-Hospital Service of the United States.

WALTER WYMAN, Surgeon-General.

HYGIENIC LABORATORY.—BULLETIN No. 16.

M. J. ROSENAU, Director

September, 1903.

THE ANTISEPTIC AND GERMICIDAL PROPERTIES OF GLYCERIN.

BY

M. J. ROSENAU.



WASHINGTON:

GOVERNMENT PRINTING OFFICE.

1903.

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No. 10.—Report upon the prevalence and geographic distribution of hookworm disease (uncinariasis or anchylostomiasis) in the United States. By Ch. Wardell Stiles.

No. 11.—Experimental investigation of *Trypanosoma lewisi*. By Edward Francis.

No. 12.—The bacteriological impurities of vaccine virus; an experimental study. By M. J. Rosenau.

No. 13.—A statistical study of the intestinal parasites of 500 white male patients at the United States Government Hospital for the Insane; by Philip E. Garrison, Brayton H. Ransom, and Earle C. Stevenson. A parasitic roundworm (*Agamomermis culicis* n. g., n. sp.) in American mosquitoes (*Culex sollicitans*); by Ch. Wardell Stiles. The type species of the cestode genus *Hymenolepis*; by Ch. Wardell Stiles.

No. 14.—Spotted fever (tick fever) of the Rocky Mountains; a new disease. By John F. Anderson.

No. 15.—Inefficiency of ferrous sulphate as an antiseptic and germicide. By Allan J. McLaughlin.

No. 16.—The antiseptic and germicidal properties of glycerin. By M. J. Rosenau.

In citing these bulletins, beginning with No. 8, bibliographers and authors are requested to adopt the following abbreviations: Bull. No. —, Hyg. Lab., U. S. Pub. Health & Mar.-Hosp. Serv., Wash., pp. —.

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United States Public Health and Marine-Hospital Service.

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THE ANTISEPTIC AND GERMICIDAL PROPERTIES OF GLYCERIN.

By MILTON J. ROSENAU, Director Hygienic Laboratory, Public Health and Marine-Hospital Service.

INTRODUCTION.

In working upon the bacteriology of vaccine virus it became evident that too much confidence was placed in the germicidal value of glycerin. In reviewing the literature upon the subject we were somewhat surprised to learn how meager has been the published work upon this important subject, especially in view of the almost universal practice of using glycerin to prepare and conserve vaccine virus and other organic matter.

Since Copeman, in 1891, devised the method of mixing glycerin with vaccine pulp, much work has been done in studying the effect of glycerin upon the microbial life contaminating bovine vaccine virus, but comparatively little upon the direct action of this interesting substance upon definite pathogenic and saprophytic bacteria. We therefore made a careful study of this question. It has engaged our attention during the odd moments of the past two years, in order to determine precisely the antiseptic and germicidal powers of this important substance. These studies are now published in detail at the request of several vaccine manufacturers, who desire to make practical use of the results obtained.

Our studies were considered under three heads:

(1) We first determined the antiseptic power of glycerin; that is, its property of restraining bacterial growth and the amount required to prevent putrefaction and fermentation.

(2) We determined its exact germicidal value; that is, the amount and time required to destroy pathogenic and saprophytic bacteria, both spore-bearing and nonspore-bearing varieties.

(3) On account of the great importance of tetanus as a contaminator of vaccine virus, we made an extensive series of special studies to determine the effect of glycerin upon the spores of this organism.

HISTORICAL.

Glycerin was discovered in 1779 by Scheele, a Swedish pharmacist, in the water which came from a simple salve. He gave it the name of "sweet principle of oils."

It is found in a free state, but exists principally in combination with the fatty acids, forming compound ethers, which constitute most of the animal and vegetable solid and liquid fats.

Pasteur showed that it is constantly found in alcoholic fermentation, it being one of the natural constituents of wine, which holds as much as 4 to 7 grams to 50 liters. Beer contains normally 2 to 3 grams per liter. (A. Catillon: *De la glycérine*. Paris, 1903.)

In 1813 Chevreul, in a memorable work, showed that fatty substances on assimilating the elements of water split up into fatty acids and glycerin. This action is brought about by the influence of alkalis, metallic oxides, hydrochloric and sulphuric acids, by water in a closed receptacle at a temperature of 220° C. (Berthelot, 1854), superheated steam, and by certain ferments, such as pancreatin (Claude Bernard).

In 1854 Berthelot demonstrated that glycerin is a triatomic alcohol, giving three series of ethers and glycerides.

Several years later Wurtz (1857) made glycerin, by synthesis, by heating on an oil bath at 120° to 125° C. for eight days tribromopropane, silver acetate, and glacial acetic acid.

GENERAL USES.

Glycerin is to-day one of the most useful substances, both in the sciences and in the arts. It is used in the preparation of pigments, for the extraction of perfumes from flowers, in lubricating oils, for the preservation of animal matter, such as fresh skins, albumin, vaccine virus, and animal extracts. Its power to protect animal matter against putrefaction, combined with its bland and nonpoisonous properties, renders it an exceedingly important and useful substance.

In pharmacy glycerin has various uses. It is used in making pill masses, in expectorant mixtures, and in the official glycerites (glycerita) of the United States Pharmacopœia.

It is used in paper making, and enters into the composition of inks, beer, wine, soaps, perfumes, toilet waters, cosmetics, and many articles of manufacture, especially explosives (nitroglycerin.)

PROPERTIES.

Glycerin, $C_6H_8O_6$ or $C_3H_4O_3$, or $C_6H_5(OH)_3$, is a colorless, sirupy liquid, without odor, and a sweetish taste. It boils at 290° C. at a pressure of 756 mm. and at 180° C. in vacuum. Heated with care it evaporates, leaving a residue, and disengages a thick vapor with an insipid but not unpleasant odor. At 150° C. it is decomposed by

losing the elements of water into less volatile compounds: Diglycerid ($C_6H_7O_5$)₂, acrolein ($C_6H_4O_2$), acetic acid, carbonic acid, and inflammable gases.

Glycerin should be neutral in reaction.

The density of the anhydrous fluid is 1.269 (1.265, Lyons), but as it is very hygroscopic it can not be kept in this state when exposed to the air without absorbing moisture, and therefore commercially the extreme point of concentration is 1.260, which corresponds to 0.03 to 0.04 per cent of water. The United States and British pharmacopœias require glycerin to have a specific gravity of 1.250; the German pharmacopœia requires 1.225 to 1.235.

Completely deprived of water by several distillations in vacuum, glycerin crystallizes into orthorhombic prisms, having a density of 1.360, fusible at 15° C.

Glycerin is soluble in all proportions in water and alcohol; insoluble in ether, chloroform, benzine, fixed oils, or volatile oils. It dissolves almost all substances soluble in water or in alcohol. Its great affinity for water is one of its best-known properties. Its properties of dissolving albumin and preventing putrefaction have special reference to its use in adding it to vaccine virus.

The number of chemical combinations with glycerin is numerous.

PREPARATION.

Glycerin is obtained by decomposing fat into its proximate constituents, either by a caustic alkali, as in the manufacture of soap, or by lead oxide, as in the preparation of lead plaster, or by the action of water at an elevated temperature under pressure. It is purified by distillation.

IMPURITIES.

Glycerin is used more or less pure, depending upon the various purposes for which it is employed. It may contain lead, iron, lime as an alkali, or sulphate, carbonate, or chlorid; oxalic, formic, or butyric acid. It is sometimes adulterated with large quantities of water, sugar, glucose, or dextrine, etc. Arsenic is also found in glycerin, particularly that which comes from the manufacture of soap. Glycerin which is obtained by treating stearine with lime is free from arsenic.

TESTS FOR IMPURITIES.

A. Catillon (*loc. cit.*, p. 20) gives the following tests for impurities in glycerin:

After testing color, odor, and taste:

1. Reaction must be exactly neutral.
2. Specific gravity, which is an evidence of the watery contents.
3. On heating 10 grams of glycerin over an alcohol lamp it should evaporate without disagreeable odor, without carbonizing, and without leaving a residue.

4. If a residue remains, add to the glycerin some distilled water and several drops of ammonia to determine the presence of lime.
5. A drop of solution of sulphhydrate of soda should not produce a color in glycerin, but throws down a black precipitate if it contains lead or iron.
6. For glucose, treat the glycerin with Fehling's solution, using the usual precautions to obtain the reduction of the copper.
7. The presence of chlorids in glycerin is indicated by silver nitrate. Pure glycerin will show neither opalescence nor precipitates.
8. Tribasic acetate of lead added to glycerin diluted with distilled water produces a limpid solution. A precipitate or a cloudiness indicates the presence of fatty acids.
9. Mix equal parts of glycerin with sulphuric acid. If carbon-dioxid gas is given off it contains oxalic or formic acid.
10. Mix the glycerin with pure alcohol and a little sulphuric acid. An odor of pineapple is immediately developed, due to butyric ether (butyric acid).
11. Glycerin heated to 120° should show no color.
12. For arsenic, test by means of Marsh's apparatus.

ACKNOWLEDGMENT.

It is a pleasure to acknowledge the faithful assistance rendered by my assistants in the laboratory in working out some of the details of this study upon glycerin, especially to Drs. John F. Anderson, T. B. McClintic, Allan J. McLaughlin, and Edward Francis.

ANTISEPTIC POWER OF GLYCERIN.

The first series of experiments were made in order to determine the antiseptic properties of glycerin; that is, its power to restrain the growth and development of bacteria. This was tested by determining the amount of glycerin necessary to add to nutrient bouillon in order to prevent putrefaction, and the amount necessary to prevent the growth and development of pure cultures of various organisms.

Erlenmayer flasks of 100 c. c. capacity were partly filled with the bouillon-glycerin solution. The flasks were then contaminated with various substances, such as rich, black garden earth, wisps of hay, or fresh stable manure.

The flasks were allowed to remain in a dark corner of the laboratory at room temperature, and results noted.

More or less growth of bacteria and mold was obtained in all the flasks containing 40 per cent of glycerin. Those containing 50 per cent or over remained clear and showed no growth. Therefore, it was plain that the antiseptic power of glycerin lies somewhere between 40 and 50 per cent.

The next series of flasks contained various percentages of glycerin between 40 and 50 and were inoculated in the same way, in order to determine more definitely the exact percentage of glycerin necessary to restrain growth and development of micro-organisms.

Nutrient bouillon containing various percentages of glycerin (X.), contaminated by the addition of garden earth, and kept in flasks at room temperature.

Percentage of glycerin.	Day upon which growth appeared.				
	Seventh day.	Eighth day.	Tenth day.	Fifteenth day.	Twenty-first day.
41 per cent.....	Fluid clear; small surface mold.				
42 per cent.....		Small surface mold.			
43 per cent.....		do.			
44 per cent.....		do.			
45 per cent.....			Small surface mold.		
46 per cent.....			do.		
47 per cent.....			do.		
48 per cent.....				Small surface mold.	
49 per cent.....					No growth.

Nutrient bouillon containing various percentages of glycerin (M.), contaminated by the addition of wisps of hay, and kept in flasks at room temperature.

Percentage of glycerin.	Day upon which growth appeared.				
	Fifth day.	Sixth day.	Seventh day.	Twelfth day.	Twenty-first day.
41 per cent.....	Feathery growth along course and adherent to straw.				
42 per cent.....	do.				
43 per cent.....		Feathery growth along course and adherent to straw.			
44 per cent.....			Very faint feathery growth along course and adherent to straw.		
45 per cent.....				Very faint growth along course and adherent to straw.	
46 per cent.....					No growth.

Nutrient bouillon containing various percentages of glycerin (P.), contaminated by the addition of stable manure, and kept in flasks at room temperature.

Percentage of glycerin.	Day upon which growth appeared.		
	Sixteenth day.	Nineteenth day.	Twenty-first day.
41 per cent.....	Small surface mold.		
42 per cent.....	do.		
43 per cent.....		Small surface mold.	
44 per cent.....			No growth.
45 per cent.....			Do.

Nutrient bouillon containing various percentages of glycerin (S.), contaminated by the addition of stable manure, and kept in flasks at room temperature.

Percentage of glycerin.	Day upon which growth appeared.			
	Ninth day.	Tenth day.	Thirteenth day.	Twenty-first day.
41 per cent	Small surface mold.			No growth. Do.
42 per cent		Small surface mold.		
43 per cent		Very small surface mold.		
44 per cent			Small surface mold.	
45 per cent				
46 per cent				

As was to be expected, the exact percentage of glycerin necessary to restrain growth varies within narrow limits with the make of glycerin used and the kind of contamination.

To sum up the antiseptic power of glycerin:

	Per cent.
Glycerin X	49
Glycerin M	45
Glycerin P	43
Glycerin S	45
Average	45.5

These results do not correspond with Miquel's work in 1884, who found that 225 grams of glycerin in 1 liter of bouillon was sufficient to prevent putrefaction.

The above test having determined the antiseptic power of glycerin against putrefactive changes, another series of experiments was made in order to determine the restraining power of glycerin against pure cultures of various micro-organisms.

The different percentages of glycerin were made as before with nutrient bouillon and this time distributed into test tubes. Each tube was inoculated with a minute but visible portion of the surface growth of the organisms, which were grown for this purpose upon agar slants under favorable conditions, and fresh young cultures were always used to make the inoculation.

The test tubes were incubated at 37° C. and examined daily for growth. In case a growth appeared it was tested for purity. The tubes were observed daily for at least eleven days and the result noted.

As a result of the foregoing experiments glycerin is found to be antiseptic in the following dilutions:

Organism.	Glycerin.				Average percent-age.
	P.	X.	S.	G.	
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
<i>Staphylococcus pyogenes aureus</i>	30	32	33	29	31
<i>Staphylococcus pyogenes citreus</i>		27	25	26	26
<i>Staphylococcus pyogenes albus</i>		27	32	31	30
<i>Staphylococcus epidermis albus</i>	27	32	28	27	28.5
<i>Bacillus typhosus</i>	25	23	25	25	24.5
<i>Bacillus coli communis</i>	28	26	24	27	26.25
<i>Bacillus icteroides</i>	26	25	23	24	24.5
<i>Bacillus acidi lactici</i>	27	25	25	25	25.5
<i>Bacillus enteritidis</i>	27	24	23	23	24.25
<i>Bacillus dysenteriae</i>	27	22	24	23	24
<i>Vibrio cholerae</i>	21	21	21	24	21.75
<i>Bacillus diphtheriae</i>	25	22	23	23	23.25
<i>Bacillus anthracis</i>	31	26	26	31	28.5
<i>Bacillus pestis</i>	23	21	21	22	21.75
<i>Bacillus glanders</i>	23	23	24	24	23.5
<i>Bacillus pyocyaneus</i>	25	22	21	25	23.25
<i>Bacillus subtilis</i>	26	24	21	27	24.5
<i>Bacillus proteus vulgaris</i>	27	22	22	21	23
<i>Bacillus megaterium</i>	20	20	15	20	18.75
<i>Bacillus prodigiosis</i>	20	21	20	20	20.25
<i>Bacillus fluorescens liquefaciens</i>	23	21	21	20	21.25
Averages	25.3	24.1	23.7	24.6	24.4

It was seen from a study of the results obtained that the antiseptic power of glycerin varies with the organism and also with the kind of glycerin used. For example, the growth of cholera and plague is retarded by the presence of 21 to 24 per cent of glycerin, while pus cocci will grow in 31 per cent. The antiseptic power of glycerin "X" and "S" is greater than that of "P" or "G."

It was also seen that the molds and common bacteria of putrefaction grow in bouillon containing between 40 and 50 per cent of glycerin, while the pure culture of the bacteria tested do not grow in percentages above 31.

Attention is especially called to the interesting fact brought out by this series of tests that the pus cocci are able to grow and multiply in higher percentages of glycerin than any of the other 18 micro-organisms tested.

THE GERMICIDAL POWER OF GLYCERIN.

The following tests were made to determine the time required for glycerin to destroy the important pathogenic organisms. The work included tests with various percentages of glycerin at different temperatures. Our object was to determine the exact effect of glycerin free from all other substances upon pure cultures. Therefore the bacteria were grown upon agar slants and carefully taken from the surface, so as to obtain the colonies free from the organic matter on which they rested.

STAPHYLOCOCCUS PYOGENES AUREUS.

The various percentages of glycerin were made with sterile distilled water and distributed into test tubes. Each tube was abundantly inoculated with a young cul-

ture of *Staphylococcus pyogenes aureus* taken from the surface of an agar slant free of foreign organic matter.

For comparison the organism was also inoculated into sterile distilled water and upon sterile slips of filter paper.

Three sets of tubes were made and kept at different temperatures, as indicated on the table.

From time to time a small quantity (about 0.5 c. c.) of the test fluids and also one of the slips of filter paper was planted in bouillon.

[+ means growth; — means no growth.]

Kept in—	Filter paper.	Dis- tilled water.	Per cent glycerin.									
			10	20	30	40	50	60	70	80	90	100
Incubator, 37° C.:												
7 days.....	+	+	—	—	+	—	—	+	—	—	+	—
13 days.....	+	—	—	—	—	—	—	—	—	—	—	—
20 days.....	+	—	—	—	—	—	—	—	—	—	—	—
27 days.....	+											
34 days.....	+											
41 days.....	+											
48 days.....	+											
55 days.....	—											
Room, 22° to 24° C.:												
7 days.....	+	+	+	+	+	+	—	+	+	+	+	—
13 days.....	+	+	+	+	—	—	—	—	—	—	—	—
20 days.....	+	+	—	—	—	—	—	—	—	—	—	—
27 days.....	+	+										
34 days.....	+	+										
41 days.....	—	—										
Ice chest, 10° to 12° C.:												
7 days.....	+	+	+	+	+	+	+	+	+	+	+	+
13 days.....	+	+	+	+	+	+	+	+	+	+	+	—
20 days.....	+	+	+	+	+	—	—	+	+	+	+	—
27 days.....	+	+	+	+	—			—	+	+	+	
34 days.....	+	+	—	—					+	+	+	
41 days.....	+	+							—	+	+	
48 days.....	+	+								—	—	
55 days.....	+	+										
62 days.....	+	+										
69 days.....	+	—										
76 days.....	+											
83 days.....	+											
90 days.....	+											
97 days.....	+											
111 days.....	—											

STAPHYLOCOCCUS EPIDERMIS ALBUS.

The various percentages of glycerin were made with sterile distilled water and distributed into test tubes. Each tube was abundantly inoculated with a young culture of *Staphylococcus epidermis albus*, taken from the surface of an agar slant, free of foreign organic matter.

For comparison the organism was also inoculated into sterile distilled water and upon sterile slips of filter paper.

Three sets of tubes were made and kept at different temperatures, as indicated on the table.

From time to time a small quantity (about 0.5 c. c.) of the test fluids and also one of the slips of filter paper were planted in bouillon.

BACILLUS COLI COMMUNIS.

From time to time a small quantity (about 0.5 c. c.) of the test fluids and also one of the slips of filter paper were planted in bouillon.

[+ means growth; - means no growth.]

[illegible]

b Material exhausted.

[+ means growth; - means no growth.]

It will be seen from these experiments that the pathogenic bacteria usually die within a week or ten days in glycerin when exposed at 37° C. in the incubator. At room temperature (22° C.) glycerin is

not so active, the organisms sometimes surviving two weeks. In the ice chest at 10° to 12° C. the germicidal power of glycerin is markedly diminished. Pus cocci live for several weeks, the colon bacillus several months.

It will be noted from these tables that 50 per cent glycerin seems to be more actively germicidal than either stronger or weaker solutions.

The next experiment was made to test the effect of glycerin upon spores, with the following results:

BACILLUS ANTHRACIS.

The various percentages of glycerin were made with sterile distilled water and distributed into test tubes. Each tube was abundantly inoculated with a growth of *B. anthracis* containing many spores taken from the surface of peptoneless agar, care being taken to carry over only a culture free from the organic matter of the media used.

For comparison the organism was also inoculated into sterile distilled water and upon sterile slips of filter paper.

Three sets of tubes were made and kept at different temperatures, as indicated on the table.

From time to time a small quantity (about 0.5 c. c.) of the test fluids, and also one of the slips of filter paper, were planted in bouillon. In addition to testing the vegetability of the organism in this manner its virulence was tested from time to time as indicated on the tables by inoculation into mice.

[+ means growth; — means no growth.]

Kept in—	Filter paper.	Distilled water.	Per cent glycerin.										Remarks.
			10	20	30	40	50	60	70	80	90	100	
Incubator, 37° C.:													
7 days	+	+	a+	+	+	+	+	+	+	+	+	+	
14 days	+	+	+	+	+	+	+	+	+	+	+	+	
61 days	+	+	+	+	+	+	+	+	+	+	+	+	
97 days	+	+	+	+	+	+	+	+	+	+	+	+	
122 days	+	+	+	+	+	+	b+	+	+	+	+	+	
153 days	+	+	+	+	+	+	e+	+	+	+	+	+	
184 days	+	+	+	+	+	+	h+	+	+	+	+	+	All cultures were of slow growth and rather scanty.
215 days	+	—	—	—	—	—	+	+	—	—	k+	—	
236 days	+	—	—	—	—	—	—	n+	—	—	—	—	
247 days	—	—	—	—	—	—	—	—	—	—	—	—	
Room, 22° to 24° C.:													
7 days	+	+	+	+	+	+	+	+	+	+	+	+	
14 days	+	+	+	+	+	+	+	+	+	+	+	+	
61 days	—	+	+	+	+	+	+	+	+	+	+	+	
97 days	—	+	+	+	—	+	+	+	+	+	+	+	
122 days	+	+	+	+	+	+	+	+	+	+	+	+	
153 days	—	+	+	+	+	+	+	f+	+	+	+	+	
184 days	—	+	+	+	+	+	+	i+	+	+	+	+	—slow and scanty growth.
215 days	—	+	—	—	—	—	—	—	—	—	l+	+	
236 days	—	+	—	—	—	—	—	—	—	—	o+	—	
247 days	—	—	—	—	—	—	—	—	—	—	—	—	
Ice chest, 10° to 12° C.:													
7 days	+	+	+	+	+	+	+	+	+	+	+	+	
12 days	+	+	+	+	+	+	+	+	+	+	+	+	
61 days	+	+	+	+	+	+	+	+	+	+	+	+	
97 days	+	+	+	+	+	+	+	+	+	+	+	+	
122 days	+	+	+	+	+	+	+	+	d+	+	+	+	
153 days	+	+	+	+	+	+	g+	+	+	+	+	+	
184 days	+	+	+	+	+	+	j+	+	+	+	+	+	—slow and scanty growth.
215 days	+	+	+	+	—	+	m+	—	+	—	+	+	
236 days	+	—	p+	+	—	+	—	—	+	—	—	—	
247 days	+	+	q+	+	—	+	+	—	—	—	+	—	
278 days	—	+	r+	—	—	+	—	—	—	—	—	—	Material exhausted.

(a) No growth first time; growth when replanted.

(b) 0.5 c. c. of the bouillon growth obtained from the 50 per cent glycerin kept in the incubator was inoculated subcutaneously in a mouse; dead in six days.

(c) 0.5 c. c. of the broth growth obtained from the 60 per cent glycerin kept at room temperature was inoculated subcutaneously in a mouse; dead in forty-eight hours.

(d) 0.5 c. c. of the broth growth obtained from the 70 per cent glycerin kept in the ice chest was inoculated subcutaneously in a mouse; dead in forty-eight hours.

(e) 1 c. c. of the bouillon growth obtained from the 50 per cent glycerin kept in the incubator was inoculated subcutaneously in a mouse; dead in forty-eight hours.

(f) 1 c. c. of the bouillon growth obtained from the 60 per cent glycerin kept at room temperature was inoculated subcutaneously in a mouse; dead in forty-eight hours.

(g) 1 c. c. of the bouillon growth obtained from the 50 per cent glycerin kept in the ice chest was inoculated subcutaneously in a mouse; dead in twenty-four hours.

(h) 1 c. c. of the bouillon growth obtained from the 50 per cent glycerin kept in the incubator was inoculated subcutaneously in a mouse; dead in seventy-two hours.

(i) 1 c. c. of the bouillon growth obtained from the 60 per cent glycerine kept at room temperature was inoculated subcutaneously in a mouse; dead in seventy-two hours.

(j) 1 c. c. of the bouillon growth obtained from the 50 per cent glycerin kept in the ice chest was inoculated subcutaneously in a mouse; dead in forty-eight hours.

(k) 1 c. c. of the bouillon growth obtained from the 90 per cent glycerin kept in the incubator was inoculated subcutaneously in a mouse; dead in eighty-four hours.

(l) 1 c. c. of the bouillon growth obtained from the 90 per cent glycerin kept at room temperature was inoculated subcutaneously in a mouse; dead in forty-eight hours.

(m) 1 c. c. of the bouillon growth obtained from the 50 per cent glycerin kept in the ice chest was inoculated subcutaneously in a mouse; dead in forty-eight hours.

(n) 1 c. c. of the bouillon growth obtained from the 60 per cent glycerin kept in the incubator was inoculated subcutaneously in a mouse; dead in sixty hours.

(o) 1 c. c. of the bouillon growth obtained from the 90 per cent glycerin kept at room temperature was inoculated subcutaneously in a mouse; dead in forty-eight hours.

(p) 1 c. c. of the bouillon growth obtained from the 10 per cent glycerin kept in the ice chest was inoculated subcutaneously in a mouse; dead in forty-eight hours.

(q) 1 c. c. of the bouillon growth obtained from the 10 per cent glycerin kept in the ice chest was inoculated subcutaneously in a mouse; dead in forty-eight hours.

(r) 1 c. c. of the bouillon growth obtained from the 10 per cent glycerin kept in the ice chest was inoculated subcutaneously in a mouse; dead in forty-eight hours.

It will be seen from this table that glycerin has practically no effect upon spores. They remain alive and virulent over two hundred days in all percentages of glycerin at temperatures of 37°, 22°, and 10° C.

The following series of tests were made to estimate more precisely the germicidal power of glycerin by counting the colonies from day to day and determining the rate of diminution.

It will be seen from these studies that glycerin produces its greatest germicidal effect during the first twenty-four hours. The more resisting members of the colonies which survive this first period are able to resist the action of the glycerin much longer when they also finally succumb.

STAPHYLOCOCCUS PYOGENES AUREUS.

The various percentages of glycerin (P.) mixed with distilled water were inoculated with a young culture of *B. staphylococcus pyogenes aureus* free of foreign organic matter and then each flask containing the test fluid was thoroughly shaken so as to make as uniform an emulsion as possible. The shaking was repeated each time before the fluid was withdrawn for the purpose of making the counts.

One series of flasks was kept in the ice chest at 10° to 12° C. and another in the incubator at 37° C.

The figures represent the number of organisms (i. e., colonies) per cubic centimeter.

When counted.	Distilled water.		20 per cent glycerin.		50 per cent glycerin.		80 per cent glycerin.		Pure glycerin.	
	Incubator.	Ice chest.	Incubator.	Ice chest.	Incubator.	Ice chest.	Incubator.	Ice chest.	Incubator.	Ice chest.
At once ..	4, 420, 000	1, 290, 000	464, 000	253, 000	64, 000	66, 000	4, 200, 000	3, 376, 000	+	+
1 day ...	104, 000	1, 050, 000	3, 000	12, 000	1, 000	22, 000	+	840, 000	+	+
2 days...	120, 000	408, 000	—	+	1, 000	12, 000	+	66, 000	—	+
3 days...	3, 000	920, 000		—	—	2, 000	+	12, 000		+
4 days...	—	110, 000				2, 500	75	+		—
5 days.....		88, 000				25	—	—		
6 days.....		60, 000				—				
8 days.....		70, 000								
12 days.....		89, 000								
17 days.....		19, 000								
22 days.....		28, 000								
27 days.....		22, 000								
31 days.....		15, 000								

When counted.	Distilled water.		50 per cent glycerin.		Pure glycerin.	
	Incubator.	Ice chest.	Incubator.	Ice chest.	Incubator.	Ice chest.
At once	4, 060	3, 900	10, 400, 000	15, 750, 000	1, 395, 000	3, 530, 000
1 day	185	140	110	1, 575, 000	100	1, 200, 000
2 days.....	1	0	0	630, 000	25	25, 200
3 days.....	1	{Bouillon+ Agar —0	0	126, 000	0	Bouillon+ Agar — 0
4 days.....	{—Bouillon —Agar 0	—Bouillon 1	—Bouillon 0	37, 800	—Bouillon 0	+Bouillon 1 +Agar (1)
5 days.....	—In bouil- lon 0	—In bouil- lon 0	—In bouil- lon 0	15, 900	—In bouil- lon 0	—In bouil- lon 0
6 days.....	0	0	0	1, 980	0	0
7 days.....				542		
8 days.....				150		
9 days.....				83		
10 days ^a				1		
12 days.....				0		
13 days.....						

^aLeft in room.

BACILLUS TYPHOSUS.

The various percentages of glycerin (S.) mixed with distilled water were inoculated with a young culture of *B. typhosus* free of foreign organic matter, and then each flask containing the test fluid was thoroughly shaken so as to make as uniform an emulsion as possible. The shaking was repeated each time before the fluid was withdrawn for the purpose of making the counts.

One series of flasks was kept in the ice chest at 10° to 12° C. and another in the incubator at 37° C.

The figures represent the number of organisms (i. e., colonies) per cubic centimeter.

When counted.	Distilled water.		20 per cent glycerin.		50 per cent glycerin.		80 per cent glycerin.		Pure glycerin.	
	Incubator.	Ice chest.	Incubator.	Ice chest.	Incubator.	Ice chest.	Incubator.	Ice chest.	Incubator.	Ice chest.
At once	10, 710, 000	11, 655, 000	180, 000	118, 000	115, 000	124, 000	2, 000	3, 000	2, 000	2, 000
1 day	5, 040, 000	21, 630, 000	150, 000	26, 000	86, 000	4, 000	1, 000	1, 000	+	+
2 days	380, 000	9, 450, 000	—	500	1, 000	1, 000	2	+	—	—
3 days	70, 000	10, 710, 000	—	300	10	26	—	—	—	—
4 days	11, 000	10, 290, 000	—	80	28	3	—	—	—	—
5 days	29	8, 400, 000	—	56	—	—	—	—	—	—
6 days	—	4, 480, 000	—	—	—	—	—	—	—	—
7 days	—	4, 410, 000	—	—	—	—	—	—	—	—
10 days	—	4, 000, 000	—	—	—	—	—	—	—	—
13 days	—	3, 860, 000	—	—	—	—	—	—	—	—
16 days	—	4, 100, 000	—	—	—	—	—	—	—	—
21 days	—	5, 080, 000	—	—	—	—	—	—	—	—
26 days	—	452, 000	—	—	—	—	—	—	—	—
31 days	—	600, 000	—	—	—	—	—	—	—	—

BACILLUS COLI COMMUNIS.

The various percentages of glycerin (M. & R.) mixed with distilled water were inoculated with a young culture of *B. coli communis* free of foreign organic matter, and then each flask containing the test fluid was thoroughly shaken so as to make as uniform an emulsion as possible. The shaking was repeated each time before the fluid was withdrawn for the purpose of making the counts.

One series of flasks was kept in the ice chest at 10° to 12° C. and another in the incubator at 37° C.

The figures represent the number of organisms (i. e., colonies) per cubic centimeter.

When counted.	Distilled water.		20 per cent glycerin.		50 per cent glycerin.		80 per cent glycerin.	
	Incubator.	Ice chest.	Incubator.	Ice chest.	Incubator.	Ice chest.	Incubator.	Ice chest.
At once	3, 760, 000	8, 064, 000	4, 440, 000	4, 680, 000	2, 416, 000	1, 760, 000	500, 000	664, 000
1 day	9, 660, 000	11, 708, 000	19, 000	131, 000	+	28, 000	+	181, 000
2 days	7, 728, 000	6, 410, 000	3, 000	56, 000	+	630, 000	+	14, 000
3 days	6, 930, 000	8, 190, 000	84	4, 200	129	28, 000	420	17, 010
4 days	5, 670, 000	9, 450, 000	4	42, 000	—	3, 000	+	111, 000
5 days	6, 130, 000	9, 210, 000	2	51, 000	—	12, 600	100	1, 000
6 days	7, 630, 000	7, 630, 000	—	10, 000	—	+	2	+
7 days	4, 330, 000	8, 400, 000	—	2, 000	—	1	—	+
8 days	4, 250, 000	9, 800, 000	—	2, 700	—	—	—	—
9 days	3, 465, 000	7, 770, 000	—	1, 000	—	—	—	—
10 days	3, 523, 000	7, 805, 000	—	100	—	—	—	—
12 days	2, 870, 000	8, 440, 000	—	100	—	—	—	—
14 days	1, 600, 000	6, 440, 000	—	40	—	—	—	—
16 days	880, 000	1, 290, 000	—	9	—	—	—	—
17 days	515, 000	6, 300, 000	—	4	—	—	—	—
19 days	480, 000	5, 230, 000	—	8	—	—	—	—
21 days	390, 000	8, 400, 000	—	—	—	—	—	—
23 days	340, 000	4, 410, 000	—	—	—	—	—	—
24 days	370, 000	4, 000, 000	—	—	—	—	—	—
26 days	280, 000	5, 200, 000	—	—	—	—	—	—
29 days	102, 000	7, 500, 000	—	—	—	—	—	—
31 days	104, 000	10, 080, 000	—	—	—	—	—	—

BACILLUS COLI COMMUNIS.

The various percentages of glycerin (P.) mixed with distilled water were inoculated with a young culture of *B. coli communis* free of foreign organic matter, and then each flask containing the test fluid was thoroughly shaken so as to make as uniform an emulsion as possible. The shaking was repeated each time before the fluid was withdrawn for the purpose of making the counts.

One series of flasks was kept in the ice chest at 10° to 12° C. and another in the incubator at 37° C.

The figures represent the number of organisms (i. e., colonies) per cubic centimeter.

When counted.	Distilled water.		20 per cent glycerin.		50 per cent glycerin.		80 per cent glycerin.		Pure glycerin.	
	Incubator.	Ice chest.	Incubator.	Ice chest.	Incubator.	Ice chest.	Incubator.	Ice chest.	Incubator.	Ice chest.
At once ..	4, 410, 000	20, 700, 000	3, 632, 000	11, 900, 000	210, 000	1, 582, 000	408, 000	400, 000	10, 000	2, 000
1 day	4, 427, 000	1, 610, 000	3, 000	5, 040, 000	+	+	4, 000	1, 000	1, 000	+
2 days ...	4, 200, 000	7, 560, 000	1	4, 550, 000	11	500, 000	—	8, 000	—	93, 000
3 days ...	18, 900, 000	9, 520, 000	—	4, 410, 000	—	15, 000		—		—
4 days ...	8, 922, 000	7, 376, 000		2, 800, 000		29, 200				
5 days ...	8, 443, 000	8, 400, 000		110, 000		16, 000				
6 days ...	7, 700, 000	8, 470, 000		8, 000		22, 000				
7 days ...	3, 500, 000	9, 500, 000		1, 000		3, 000				
8 days ...	Flask broken.	11, 000, 000		600		900				
9 days ...		12, 000, 000		18		200				
11 days ...		14, 000, 000		24		4				
13 days ...		17, 000, 000		15		—				
15 days ...		14, 280, 000		5						
16 days ...		10, 710, 000		2						
18 days ...		13, 860, 000		—						
20 days ...		10, 290, 000								
24 days ...		11, 600, 000								
27 days ...		9, 480, 000								
31 days ...		8, 640, 000								

BACILLUS DIPHTHERIÆ.

The various percentages of glycerin (P.) mixed with distilled water were inoculated with a young culture of *B. diphtheriæ* free of foreign organic matter, and then each flask containing the test fluid was shaken so as to make as uniform an emulsion as possible. The shaking was repeated each time before the fluid was withdrawn for the purpose of making the counts.

One series of flasks was kept in the ice chest at 10° to 12° C. and another in the incubator at 37° C.

The figures represent the number of organisms (i. e., colonies) per cubic centimeter.

When counted.	Distilled water.		50 per cent glycerin.		100 per cent glycerin.		Remarks.
	Incubator.	Ice chest.	Incubator.	Ice chest.	Incubator.	Ice chest.	
At once	1, 080	2, 450	10		20	10	All sterile in bouillon.
1 day	0	0	1	0	0	1	
2 days	0	0	Agar. 0	0	0	Agar. 0	In bouillon.

It is again evident from these experiments that the pus cocci in pure culture are destroyed by the action of glycerin within two weeks.

Glycerin seems to be an active poison for the bacillus of diphtheria throughout all our experiments.

The organisms belonging to the typhoid and colon group sometimes show a more marked resistance in some of our other tests than the preceding.

Pus from axillary abscess containing *Staphylococcus pyogenes aureus* in pure culture, planted in varying percentages of glycerin (G) in distilled water and kept in the incubator at 37° C. From time to time small portions of the emulsion were drawn off and planted upon agar and in bouillon.

Days.	Percentage of glycerin.										Remarks.
	10	20	30	40	50	60	70	80	90	100	
7.....	+	+	+	-	-	-	-	-	-	-	All marked — gave no growth in broth.
14.....	+	+	+	-	-	-	-	-	-	-	
21.....	+	+	+	.	.	.	.	.	.	.	
28.....	-	+	-	.	.	.	.	.	.	.	
35.....	+	+	-	.	.	.	.	.	.	.	
42.....	+	-	-	.	.	.	.	.	.	.	
49.....	-	-	-	.	.	.	.	.	.	.	

STAPHYLOCOCCUS IN PURE CULTURE FROM ABOVE PUS.

A pure culture of *Staphylococcus pyogenes aureus* isolated from the above pus obtained from axillary abscess was planted in various percentages of glycerin and tested as above.

Days.	Percentage of glycerin.										Remarks.
	10	20	30	40	50	60	70	80	90	100	
7.....	+	+	+	-	+	-	-	-	-	-	All marked - gave no growth in broth.
14.....	+	+	+		-						
21.....	+	+	+		-						
28.....	+	+	+		-						
35.....	-	+	-		-						
42.....	-	-	-		-						

Pus from an acute abscess of the face, containing *Staphylococcus pyogenes aureus* in pure culture, planted in varying percentages of glycerin (G) in distilled water and kept in the incubator at 37° C. From time to time small portions of the emulsion were drawn off and planted upon agar and in bouillon.

[illegible]

STAPHYLOCOCCUS IN PURE CULTURE FROM ABOVE PUS.

A pure culture of *Staphylococcus pyogenes aureus* isolated from the above pus obtained from acute abscess of the face was planted in various percentages of glycerin and tested as above.

Days.	Percentage of glycerin.										Remarks.
	10	20	30	40	50	60	70	80	90	100	
7.....	+	+	+	-	-	-	-	-	-	-	All marked — gave no growth in broth.
14.....	+	+	+	.	.	.	.	.	.	.	
21.....	+	+	+	.	.	.	.	.	.	.	
28.....	+	+	+	.	.	.	.	.	.	.	
35.....	-	+	-	.	.	.	.	.	.	.	
42.....	-	-	-	.	.	.	.	.	.	.	

Pus from a bone felon of the finger, containing *Staphylococcus pyogenes albus* in pure culture, planted in various percentages of glycerin (G) in distilled water and kept in the incubator at 37° C. From time to time small portions of the emulsion were drawn off and planted upon agar and in bouillon.

Days.	Percentage of glycerin.										Remarks.
	10	20	30	40	50	60	70	80	90	100	
7.....	+	+	+	—	—	—	—	—	—	—	All marked — gave no growth in broth.
14.....	+	+	+	—	—	—	—	—	—	—	
21.....	+	+	+	—	—	—	—	—	—	—	
28.....	+	+	+	—	—	—	—	—	—	—	
35.....	+	—	—	—	—	—	—	—	—	—	
42.....	—	—	—	—	—	—	—	—	—	—	

STAPHYLOCOCCUS IN PURE CULTURE FROM ABOVE PUS.

A pure culture of *Staphylococcus pyogenes albus* isolated from the above pus obtained from a bone felon of the finger was planted in various percentages of glycerin and tested as above.

Days.	Percentage of glycerin.										Remarks.
	10	20	30	40	50	60	70	80	90	100	
7.....	+	+	+	—	—	—	—	—	—	—	All marked — gave no growth in broth.
14.....	+	+	+	—	—	—	—	—	—	—	
21.....	+	+	+	—	—	—	—	—	—	—	
28.....	+	+	+	—	—	—	—	—	—	—	
35.....	+	+	+	—	—	—	—	—	—	—	
42.....	+	—	—	—	—	—	—	—	—	—	
49.....	+	—	—	—	—	—	—	—	—	—	
56.....	—	—	—	—	—	—	—	—	—	—	

From these experiments it will be seen that glycerin has the power of destroying pus organisms whether in pure culture or in the pus itself within two weeks when exposed at these temperatures. As these tests were made in the incubator at 37° C. they can not be taken as an evidence of what glycerin may always do in vaccine virus when kept cool.

TETANUS IN GLYCERIN.

VIABILITY OF TETANUS IN GLYCERIN.

On January 2, 1903, tetanus was planted into 1,000 c. c. of ordinary bouillon and grown two weeks in a Novy jar at 37° C. Examination of the growth showed that it was a pure culture. The spores and toxin were then separated by filtration and the toxin was set aside.

The spores were diluted with distilled water and filtered. The residue was again suspended in water and filtered several times in order to remove the toxin. 1,000 c. c. were used in the washing.

The toxin and the watery suspension of spores were then tested separately on mice as indicated by the following table:

Result.

Inoculated into mice—	Tetanus toxin			Tetanus spores.		
Jan. 22: 0.00006 c. c. .0002 c. c. .0004 c. c.	Jan. 23-27, N. Jan. 23, N.... Jan. 23, N....	Jan. 28-30, P. Jan. 24, P ... Jan. 24-28, P.	Recovered.. Jan. 25, dead Jan. 29, dead	Jan. 23, N. Jan. 23, N. Jan. 23, N.	Jan. 24-30, P. Jan. 24-31, P. Jan. 24-29, P.	Jan. 31, recovered. Jan. 31, killed. Jan. 30, killed.

Six sets of tubes were prepared, each set composed of eleven tubes, each tube containing 10 c. c. of fluid. The first tube of each set contained distilled water; the second contained 10 per cent pure glycerin in distilled water; the other tubes contained 20 per cent, 30 per cent, etc., to 100 per cent. 0.5 c. c. of toxin was added to each tube of three sets and the suspension of spores was distributed evenly among the other three sets, each tube getting 0.5 c. c. A set of spores and a set of toxin were placed at room temperature; a set of each was kept in the cool chamber and the other two sets were put in the incubator at 37° C.

On February 1 these tubes were tested on mice as shown in the following tables, having been two days in glycerin, and thereafter each month.

TETANUS SPORES IN GLYCERIN.

The following tables give the results of our studies of tetanus spores, washed free of toxin, in varying percentages of glycerin, and at different temperatures:

Tetanus spores.

AFTER TWO DAYS' EXPOSURE TO GLYCERIN.

[+ = typical symptoms of tetanus; — = no symptoms of tetanus; k = killed; d = died.]

Quantity inoculated into mice, percentages of glycerin, and temperature.	Result.											
	1st day.	2d day.	3d day.	4th day.	5th day.	6th day.	7th day.	8th day.	9th day.	10th day.	11th day.	12th day.
Incubator, 37° C.:												
0.05 c. c. distilled water	—	+	+	+k								
20 per cent glycerin.....	—	+	+k									
40 per cent glycerin.....	—	+	+	+k								
60 per cent glycerin.....	—	+	+k									
80 per cent glycerin.....	—	+	+	+k								
100 per cent glycerin.....	—	—	+	+	+	+	+	+	+	+	+	
Ice chest, 10° to 12° C.:												
0.05 c. c. distilled water	—	+	+	+k								
20 per cent glycerin.....	+	+	+k									
40 per cent glycerin.....	—	+	+k									
60 per cent glycerin.....	—	+	+k									
80 per cent glycerin.....	—	+	+	+k								
100 per cent glycerin.....	—	+	+	+k								
Room, about 20° C.:												
0.02 c. c. distilled water	—	—	+	+	+	+k						
20 per cent glycerin.....	—	+	+	+	+k							
40 per cent glycerin.....	—	—	+	+	+	+k						
60 per cent glycerin.....	—	+	+	+	+k							
80 per cent glycerin.....	—	+	+	+	+k							
100 per cent glycerin.....	—	+	+	+	+	+k						

[+ = typical symptoms of tetanus; - = no symptoms of tetanus; k = killed; d = died.]

AFTER SIXTY DAYS' EXPOSURE TO GLYCERIN.

AFTER NINETY DAYS' EXPOSURE TO GLYCERIN.

[illegible]

The spores kept in various percentages of glycerin in the incubator have apparently died out, viz, having failed to produce symptoms when inoculated directly into mice. Tests were now made (May 9) to see whether these spores were really dead or whether their virulence was simply attenuated. Small quantities from each of the test dilutions were therefore inoculated into freshly prepared bouillon and grown anærobically in a Novy jar in an atmosphere of hydrogen plus pyrogallic acid and caustic potash, with the following results:

Planted May 9.

Mixture.	Result.
Aqueous solution.....	Growth, no spores, contaminated.
10 per cent glycerin	Mixed, no spores, contaminated.
20 per cent glycerin	No growth.
30 per cent glycerin	Many spores.
40 per cent glycerin	Many spores and rods.
50 per cent glycerin	Many spores and rods.
60 per cent glycerin	Many spores.
70 per cent glycerin	Many spores.
80 per cent glycerin	Many spores.
90 per cent glycerin	Spores and many rods.
100 per cent glycerin	Many spores.

A few drops of the growths thus obtained were on May 21 inoculated into mice, with the result that all died within a few hours without showing characteristic symptoms. The inoculations were repeated June 3 (i. e., 25 days' growth in bouillon), 0.0005 c. c. was inoculated subcutaneously into the flank of each mouse, with the following results:

[+ = typical symptoms of tetanus; -- = no symptoms of tetanus; k = killed; d = died.]

Quantity inoculated into mice, percentage of glycerin, and temperature.	Result.											
	1st day.	2d day.	3d day.	4th day.	5th day.	6th day.	7th day.	8th day.	9th day.	10th day.	11th day.	12th day.
Incubator:												
0.0005 c. c. distilled water	+k											
10 per cent glycerin.....	+k											
20 per cent glycerin....	-a											
30 per cent glycerin....	+k											
40 per cent glycerin....	+k											
50 per cent glycerin....	+k											
60 per cent glycerin....	+k											
70 per cent glycerin....	+k											
80 per cent glycerin....	+d											
90 per cent glycerin....	+d											
100 per cent glycerin....	+d											

a. This showed no growth. Therefore about 0.25 c. c. of the dilution was injected into a mouse. No symptoms.
The dilutions, etc., for the others were as follows: 10 c. c. aq. + 0.1 c. c. spores = 1:100. 0.05 c. c. of this solution would contain 1/2000 of the original, or .0005.

Showing that although the spores had lost their power of producing tetanus when inoclated directly into mice, they were not dead, as they regained their original activity and virulence when reactivated by growing under favorable conditions.

Tetanus spores.

AFTER ONE HUNDRED AND TWENTY DAYS' EXPOSURE TO GLYCERIN.

[+ = typical symptoms of tetanus; -- = no symptoms of tetanus; k = killed; d = died.]

Quantity inoculated into mice, per-centage of glycerin, and tempera-ture.	Result.											
	1st day.	2d day.	3d day.	4th day.	5th day.	6th day.	7th day.	8th day.	9th day.	10th day.	11th day.	12th day.
Incubator, 37° C.:												
0.05 c. c. distilled water.....	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
20 per cent glycerin.....	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
40 per cent glycerin.....	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
60 per cent glycerin.....	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
80 per cent glycerin.....	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
100 per cent glycerin.....	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Ice chest, 10° to 12° C.:												
0.05 c. c. distilled water.....	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
20 per cent glycerin.....	—	+	+k	-----	-----	-----	-----	-----	-----	-----	-----	-----
40 per cent glycerin.....	—	+	+k	-----	-----	-----	-----	-----	-----	-----	-----	-----
60 per cent glycerin.....	—	?	+	—	+	+k	-----	-----	-----	-----	-----	-----
80 per cent glycerin.....	—	—	—	—	—	—	—	—	—	—	—	—
100 per cent glycerin.....	—	—	—	—	—	—	—	—	—	—	—	—
Room, about 20° C.:												
0.05 c. c. distilled water.....	—	—?	—	—	—	—	—	—	—	—	—	—
20 per cent glycerin.....	—	—	—	—	—	—	—	—	—	—	—	—
40 per cent glycerin.....	—	—	—	—	—	—	—	—	—	—	—	—
60 per cent glycerin.....	—	—	—	—	—	—	—	—	—	—	—	—
80 per cent glycerin.....	—	—	—	—	—	—	—	—	—	—	—	—
100 per cent glycerin.....	—	—	—	—	—	—	—	—	—	—	—	—

AFTER ONE HUNDRED AND FIFTY DAYS' EXPOSURE TO GLYCERIN.

Ice chest, 10° to 12° C.:												
0.05 c. c. 10 per cent glycerin.....	—	+	+	+k	-----	-----	-----	-----	-----	-----	-----	-----
30 per cent glycerin.....	—	+k	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
50 per cent glycerin.....	—	+k	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
70 per cent glycerin.....	—	—	+	+	+	+k	-----	-----	-----	-----	-----	-----
90 per cent glycerin.....	—	—	—	—	—	—	—	—	—	—	—	—
Room, about 20° C.:												
0.05 c. c. 10 per cent glycerin.....	—	—	—	—	—	—	—	—	—	—	—	—
30 per cent glycerin.....	—	—	+	+	+	+k	-----	-----	-----	-----	-----	-----
50 per cent glycerin.....	—	—	—	—	—	—	—	—	—	—	—	—
70 per cent glycerin.....	—	—	—	—	—	—	—	—	—	—	—	—
90 per cent glycerin.....	—	—	—	—	—	—	—	—	—	—	—	—

AFTER ONE HUNDRED AND EIGHTY DAYS' EXPOSURE TO GLYCERIN.

Ice chest, 10° to 12° C.:												
0.05 c. c. 20 per cent glycerin.....	—	?	+k	-----	-----	-----	-----	-----	-----	-----	-----	-----
40 per cent glycerin.....	—	+k	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
60 per cent glycerin.....	—	+	+k	-----	-----	-----	-----	-----	-----	-----	-----	-----
80 per cent glycerin.....	—	—	—	—	—	—	—	—	—	—	—	—
100 per cent glycerin.....	—	—	—	—	—	—	—	—	—	—	—	—
Room, about 20° C.:												
0.05 c. c. 20 per cent glycerin.....	—	—	—	—	—	—	—	—	—	—	-----	-----
40 per cent glycerin.....	—	—	—	—	—	—	—	—	—	—	-----	-----
60 per cent glycerin.....	—	—	?	+k	-----	-----	-----	-----	-----	-----	-----	-----
80 per cent glycerin.....	—	—	—	—	—	—	—	—	—	—	-----	-----
100 per cent glycerin.....	—	—	—	—	—	—	—	—	—	—	-----	-----

We learn from these studies that tetanus spores may die within thirty days in glycerin at the body temperature; but they live for months (one hundred and eighty days) at room temperature or in the ice chest. The virulence of the spores is generally lost long before their power of growing and multiplying if placed under favorable conditions.

TETANUS TOXIN IN GLYCERIN.

A series of tests was then made to determine the effect of glycerin upon tetanus toxin.

In each instance a quantity of the glycerin containing 0.0025 c. c. of the toxin was inoculated into the flank of a mouse and observed for symptoms. This quantity was over ten times the M. L. D.

It was found that the toxin exposed to the glycerin gradually lost its virulence after sixty days at the body temperature, but that it was still active after one hundred and eighty days in the ice chest or at room temperature.

If vaccine virus, therefore, was contaminated with such toxin the glycerin would have no influence upon it.

VIABILITY OF MIXED CULTURE OF TETANUS IN VARIOUS PERCENTAGES OF GLYCERIN.

This culture was the same as was used for testing the viability of the mixed culture of tetanus on dry points and in glycerinated virus.^a It was planted into 400 c. c. of bouillon on June 25 and kept at 37° for two weeks in a Novy jar. It contained a coccus, a slender motile rod of good length, which contained a central oval spore, in addition to a rich growth of tetanus organisms.

On July 14 the 400 c. c. were subjected to a Pasteur filter to remove the toxins. The residue was washed several times with distilled water to remove any trace of toxins from the tetanus organisms. The excess of water in which the tetanus remained suspended was evaporated in a vacuum containing a vessel of calcium chloride until it reached a bulk of about 2 c. c.

Three series of test tubes were prepared. Each series represented ten different percentages of pure glycerin in water, namely, 10, 20, 30 per cent, etc., to 100 per cent. Into each of the thirty tubes comprising the three series we put an equal amount of the watery suspension of mixed tetanus organisms on July 16 and paraffined the cotton plugs. The three series were then put at different temperatures—one at room temperature, about 20° C.; another in the incubator at 37°, and the other in the ice chest at 10° to 12° C. Before placing the series at different temperatures, however, they were tested on mice.

As a control, inoculations into mice were made from three tubes selected at random, which were the 10 per cent incubator, 30 per cent ice chest, and 60 per cent room temperature. The three series were then placed in their appropriate temperatures and tested on mice at intervals.

It was found that mixed cultures of tetanus in glycerin at the body

^aSee Bulletin No. 12.—The bacteriological impurities of vaccine virus; an experimental study. By M. J. Rosenau.

heat began to lose their virulence in thirty days, but at room temperature were virulent for mice at the end of ninety days, and in the ice chest at one hundred and sixty-five, when all of the material was exhausted.

RÉSUMÉ AND CONCLUSIONS.

This study was undertaken and is published on account of its importance from a public-health standpoint, particularly in view of the fact that glycerin is used to conserve vaccine virus and analogous products. On account of its bland and nonpoisonous properties glycerin has long been used as a preservative for organic matter; but not until 1891, when Copeman claimed for it special virtues as a germicide, did it come into general use to purify vaccine virus.

A false sense of security arose owing to an overestimate of the antiseptic and germicidal value of glycerin. This fact we have brought out in previous publications on the subject of the bacteriological impurities of vaccine virus. Other substances, such as chloroform vapor, chloretone, potassium cyanide, carbolic acid, formalin, etc., have since been used as a substitute for glycerin with more or less success, and it is possible that one of these more energetic germicidal substances may be found to be superior to glycerin for this particular purpose in commercial practice.

The experiments are published in detail at the request of several vaccine producers who desire to know the exact value of glycerin as a germicide and antiseptic.

In brief, it may be stated that glycerin has distinct but very feeble germicidal and antiseptic properties. The results are summarized as follows:

GLYCERIN AS AN ANTISEPTIC.

Small quantities of glycerin, less than 10 per cent, added to nutrient media have well-known powers of favoring the growth and multiplication of many forms of bacteria.

The presence of 50 per cent of glycerin will restrain all bacterial growth. Less than this amount can not be depended upon for the preservation of vaccines and other organic growths.^a

The antiseptic power varies for the different glycerins. For instance, some restrain all growth and development when present in the proportion of 43 per cent; others require 49 per cent.

No evident growth or multiplication of bacteria takes place in nutrient media containing 32 per cent of glycerin, but molds grow in stronger percentages, viz, 40 to 49 per cent.

In order to prevent the growth and development of pus cocci at least 33 per cent of glycerin must be present. This is more than that

^a The percentages throughout this paper are by volumes.

required to restrain the growth and multiplication of the other eighteen different pathogenic and saprophytic bacteria tested.

GLYCERIN AS A GERMICIDE.

Glycerin has a distinct, though exceedingly feeble, germicidal action. It probably acts by virtue of its great affinity for water, abstracting this substance from the germ.

As a rule, glycerin destroys the micrococci of suppuration, whether these be in pure culture or in the pus itself, within two weeks. This action, like that of all germicides, depends for its activity upon the temperature. Pus cocci may live in glycerin for months in the ice chest. They would die in a week at the body temperature.

Glycerin seems to be a selective poison for the bacillus of diphtheria, which in all of our experiments died much more quickly than any of the other organisms tested.

The bacteria of the typhoid and colon group often show a marked resistance to the effects of glycerin in strong proportions.

Glycerin asserts its greatest germicidal effect during the first twenty-four hours. The remaining members of the colonies which resist its action for this first period succumb very slowly.

Glycerin in all proportions has practically no effect upon endogenous spores. We have kept anthrax spores alive and virulent two hundred days in the stronger percentages and at warm temperatures.

TETANUS IN GLYCERIN.

Tetanus spores in pure culture, freed of all organic matter and washed free of toxin, may lose their virulence in glycerin in thirty days at the body temperature, but they live for months (one hundred and eighty days) at room temperature or in the ice chest. Glycerin, therefore, can not be depended upon to purify vaccine or other organic matter containing this contamination. The virulence of the spores is lost long before they actually die, for they still retain the power of growing and multiplying if placed under favorable conditions. Under these circumstances they also regain their original pathogenic properties.

Glycerin has practically no effect upon tetanus toxin. We found such toxin added to glycerin to be active for sixty days at the body temperature and one hundred and eighty days at room temperature or in the ice chest.

